

## AMENDMENT TO THE CLAIMS

1. (Currently Amended) An isolated nucleic acid molecule encoding a *Herpesviridae* thymidine kinase enzyme comprising at least one mutation in the Q substrate binding domain, wherein said domain is selected from the group consisting of: SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, and SEQ ID NO:121, and wherein said mutation alters the substrate specificity of said thymidine kinase, as compared to unmutated thymidine kinase.

2. (Currently amended) An isolated nucleic acid molecule encoding a *Herpesviridae* thymidine kinase enzyme of SEQ ID NO:1 comprising at least three mutations relative to SEQ ID NO:1, at least two of said mutations encoding amino acid substitutions that are located one, two or three amino acids toward the N-terminus from a DRH nucleoside binding site, wherein said DRH binding site consists of Asp at position 392, Arg at position 393, and His at position 394 of the amino acid sequence encoded by SEQ ID NO:1, and at least one of said mutations encoding an amino acid substitution that is located four or five amino acids toward the C-terminus from ~~a~~said DRH nucleoside binding site, wherein said mutations increase a biological activity of said thymidine kinase, as compared to unmutated thymidine kinase.

3. (Previously Presented) The isolated nucleic acid molecule of claim 1, further comprising at least one mutation that is an amino acid substitution within a DRH nucleoside binding site.

4. (Previously Presented) The isolated nucleic acid molecule of claim 1, further comprising at least one mutation that is an amino acid substitution located 4, 5 or 6 amino acids toward the C-terminus from a DRH nucleoside binding site.

5. (Previously Presented) The isolated nucleic acid molecule encoding a thymidine kinase enzyme according to claim 1 further comprising at least one mutation that encodes an amino acid substitution located from 1 to 7 amino acids toward the N-terminus from the DRH nucleoside binding site.

6. (Cancelled)

7. (Currently amended) The isolated nucleic acid molecule encoding a thymidine kinase enzyme according to claim 1 or 2 wherein said enzyme is truncated or contains an in-frame deletion.

8. (Currently amended) The isolated nucleic acid molecule encoding a thymidine kinase enzyme according to claim 1 or 2 wherein said thymidine kinase enzyme is capable of phosphorylating a nucleoside analogue at least one-fold over the phosphorylation of the nucleoside analogue by a wild-type thymidine kinase enzyme.

9. (Previously Presented) The isolated nucleic acid molecule according to claim 8 wherein said nucleoside analogue is selected from the group consisting of ganciclovir, acyclovir, trifluorothymidine, 1-[2-deoxy, 2-fluoro, beta-D-arabino furanosyl]-5-iodouracil, ara-A, araT 1-beta-D-arabinofuranoxyl thymine, 5-ethyl-2'-deoxyuridine, 5-iodo-5'-amino-2,5'-dideoxyuridine, idoxuridine, AZT, AIU, dideoxycytidine and AraC.

10. (Currently amended) The isolated nucleic acid molecule encoding a thymidine kinase enzyme according to claim 1 or 2 wherein said thymidine kinase enzyme is capable of phosphorylating a nucleoside analogue, and wherein

$$z < \left[ \frac{(\text{TK}_m \text{NA}_p)/(\text{TK}_m \text{T}_p)}{(\text{TK}_{wt} \text{NA}_p)/(\text{TK}_{wt} \text{T}_p)} \right]$$

wherein  $\text{TK}_m \text{NA}_p$  is the rate of phosphorylation of a nucleoside analogue by a thymidine kinase mutant,  $\text{TK}_m \text{T}_p$  is the rate of phosphorylation of thymidine by a thymidine kinase mutant,  $\text{TK}_{wt} \text{NA}_p$  is the rate of phosphorylation of a nucleoside analogue by an unmutated thymidine kinase enzyme,  $\text{TK}_{wt} \text{T}_p$  is the rate of

phosphorylation of a thymidine kinase enzyme by an unmutated thymidine kinase enzyme, and z is selected from the group consisting of 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5.

11. (Previously Presented) The isolated nucleic acid molecule according to claim 10 wherein said nucleoside analogue is selected from the group consisting of ganciclovir, acyclovir, trifluorothymidine, 1-[2-deoxy, 2-fluoro, beta-D-arabino furanosyl]-5-iodouracil, ara-A, araT 1-beta-D-arabinofuranoxyl thymine, 5-ethyl-2'-deoxyuridine, 5-iodo-5'-amino-2,5'-dideoxyuridine, idoxuridine, AZT, AIU, dideoxycytidine and AraC.

12. (Currently amended) An expression vector comprising a promoter operably linked to a nucleic acid molecule according to any one of claim 1 or 2.

13. (Previously Presented) The expression vector according to claim 12 wherein said promoter is selected from the group consisting of MoMLV LTR, Cytomegalovirus Immediate Early Promoter, and Cytomegalovirus Immediate Late Promoter.

14. (Previously Presented) The expression vector according to claim 13 wherein said promoter is a tissue-specific promoter.

15. (Previously Presented) The expression vector according to claim 14 wherein said tissue-specific promoter is selected from the group consisting of the tyrosine hydroxylase promoter, adipocyte P2 promoter, PEPCK promoter,  $\alpha$  fetoprotein promoter, whey acidic promoter, and casein promoter.

16. (Withdrawn) An isolated nucleic acid molecule encoding a fusion protein comprising a guanylate kinase moiety and a thymidine kinase moiety, wherein said fusion protein possesses a biological activity of guanylate kinase and a biological activity of thymidine kinase, wherein said thymidine kinase moiety is either a

*Herpesviridae* thymidine kinase or a mutant *Herpesviridae* thymidine kinase that possesses an increased biological activity, compared with unmutated thymidine kinase.

17. (Withdrawn) The isolated nucleic acid molecule according to claim 16, wherein at least one of said guanylate kinase moiety and said thymidine kinase moiety is truncated.

18. (Withdrawn) The isolated nucleic acid molecule according to claim 16, wherein said guanylate kinase moiety is a mammalian guanylate kinase.

19. (Withdrawn) The isolated nucleic acid molecule according to claim 18, wherein said mammalian guanylate kinase moiety is a murine guanylate kinase or a human guanylate kinase.

20. (Withdrawn) The isolated nucleic acid molecule according to claim 16, wherein said mutant thymidine kinase is an enzyme comprising one or more mutations, at least one of said mutations encoding an amino acid substitution located toward the N-terminus from a DRH nucleoside binding site.

21. (Withdrawn) The isolated nucleic acid molecule according to claim 16, wherein said mutant thymidine kinase is an enzyme comprising one or more mutations, at least one of said mutations being an amino acid substitution within a DRH nucleoside binding site.

22. (Withdrawn) The isolated nucleic acid molecule according to claim 16, wherein said mutant thymidine kinase is an enzyme comprising at least three mutations, at least two of said mutations encoding amino acid substitutions that are located one, two or three amino acids toward the N-terminus from a DRH nucleoside binding site, and at least one of said mutations encoding an amino acid substitution that is located four or five amino acids toward the C-terminus from a DRH nucleoside binding site.

23. (Withdrawn) The isolated nucleic acid molecule according to claim 20, wherein said mutant thymidine kinase is an enzyme comprising at least one mutation in the Q substrate binding domain.

24. (Withdrawn) The isolated nucleic acid molecule according to claim 16, wherein said thymidine kinase is selected from the group consisting of Herpes Simplex Virus Type 1 thymidine kinase and Herpes Simplex Virus Type 2 thymidine kinase.

25. (Withdrawn) An expression vector comprising the isolated nucleic acid molecule of claim 16.

26. (Withdrawn) The expression vector of claim 25 further comprising a promoter operably linked to said nucleic acid molecule.

27. (Withdrawn) A viral vector capable of directing the expression of a nucleic acid molecule according to any one of claims 1-11 and 16.

28. (Withdrawn) The viral vector according to claim 27 wherein said vector is selected from the group consisting of herpes simplex viral vectors, adenoviral vectors, adenovirus-associated viral vectors and retroviral vectors.

29. (Withdrawn) Host cells carrying a vector according to claim 27.

30. (Withdrawn) The host cells according to claim 29 wherein said cells are selected from the group consisting of human cells, dog cells, monkey cells, rat cells, and mouse cells.

31. (Withdrawn) An isolated *Herpesviridae* thymidine kinase enzyme comprising at least three mutations, at least two of said mutations encoding amino acid

substitutions that are located one, two or three amino acids toward the N-terminus from a DRH nucleoside binding site, and at least one of said mutations encoding an amino acid substitution that is located four or five amino acids toward the C-terminus from a DRH nucleoside binding site, wherein said mutations increase a biological activity of said thymidine kinase, as compared to unmutated thymidine kinase.

32. (Withdrawn) An isolated *Herpesviridae* thymidine kinase enzyme comprising at least one mutation in the Q substrate binding domain, wherein said mutation increases a biological activity of said thymidine kinase, as compared to unmutated thymidine kinase.

33. (Withdrawn) The isolated *Herpesviridae* thymidine kinase enzyme of claim 32, further comprising at least one mutation that is an amino acid substitution within a DRH nucleoside binding site.

34. (Withdrawn) The isolated *Herpesviridae* thymidine kinase enzyme of claim 32, further comprising at least one mutation that is an amino acid substitution located 4, 5 or 6 amino acids toward the C-terminus from a DRH nucleoside binding site.

35. (Withdrawn) The isolated *Herpesviridae* thymidine kinase enzyme according to claim 32 further comprising at least one mutation that encodes an amino acid substitution located from 1 to 7 amino acids toward the N-terminus from the DRH nucleoside binding site.

36. (Withdrawn) The isolated *Herpesviridae* thymidine kinase enzyme according to any one of claims 31-35, wherein said thymidine kinase is selected from the group consisting of Herpes Simplex Virus Type 1 thymidine kinase and Herpes Simplex Virus Type 2 thymidine kinase.

37. (Withdrawn) The isolated *Herpesviridae* thymidine kinase enzyme according to claims 31 or 32 wherein said enzyme is truncated or contains an in-frame deletion.

38. (Withdrawn) The isolated *Herpesviridae* thymidine kinase enzyme according to claims 31 or 32 wherein said thymidine kinase enzyme is capable of phosphorylating a nucleoside analogue at least one-fold over the phosphorylation of the nucleoside analogue by a wild-type thymidine kinase enzyme.

39. (Withdrawn) The isolated *Herpesviridae* thymidine kinase enzyme according to claim 38 wherein said nucleoside analogue is selected from the group consisting of ganciclovir, acyclovir, trifluorothymidine, 1-[2-deoxy, 2-fluoro, beta-D-arabino furanosyl]-5-iodouracil, ara-A, araT 1-beta-D-arabinofuranoxyl thymine, 5-ethyl-2'-deoxyuridine, 5-iodo-5'-amino-2,5'-dideoxyuridine, idoxuridine, AZT, AIU, dideoxycytidine and AraC.

40. (Withdrawn) The *Herpesviridae* thymidine kinase enzyme according to claims 31 or 32 wherein said thymidine kinase enzyme is capable of phosphorylating a nucleoside analogue, and wherein

$$z < \left[ \frac{(\text{TK}_m \text{NA}_p)/(\text{TK}_m \text{T}_p)}{(\text{TK}_{wt} \text{NA}_p)/(\text{TK}_{wt} \text{T}_p)} \right]$$

wherein  $\text{TK}_m \text{NA}_p$  is the rate of phosphorylation of a nucleoside analogue by a thymidine kinase mutant,  $\text{TK}_m \text{T}_p$  is the rate of phosphorylation of thymidine by a thymidine kinase mutant,  $\text{TK}_{wt} \text{NA}_p$  is the rate of phosphorylation of a nucleoside analogue by an unmutated thymidine kinase enzyme,  $\text{TK}_{wt} \text{T}_p$  is the rate of phosphorylation of a thymidine kinase enzyme by an unmutated thymidine kinase enzyme, and  $z$  is selected from the group consisting of 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5.

41. (Withdrawn) The *Herpesviridae* thymidine kinase enzyme according to claim 40 wherein said nucleoside analogue is selected from the group consisting of ganciclovir, acyclovir, trifluorothymidine, 1-[2-deoxy, 2-fluoro, beta-D-arabino furanosyl]-5-iodouracil, ara-A, araT 1-beta-D-arabinofuranoxyl thymine, 5-ethyl-2'-deoxyuridine, 5-iodo-5'-amino-2,5'-dideoxyuridine, idoxuridine, AZT, AIU, dideoxycytidine and AraC.

42. (Withdrawn) A fusion protein comprising a guanylate kinase moiety and a thymidine kinase moiety, wherein said fusion protein possesses a biological activity of said guanylate kinase and a biological activity of said thymidine kinase, wherein said thymidine kinase moiety is either a *Herpesviridae* thymidine kinase or a mutant *Herpesviridae* thymidine kinase that possesses an increased biological activity, compared with unmutated thymidine kinase.

43. (Withdrawn) The fusion protein according to claim 42, wherein at least one of said guanylate kinase moiety and said thymidine kinase moiety is truncated.

44. (Withdrawn) The fusion protein according to claim 42, wherein said guanylate kinase moiety is a mammalian guanylate kinase.

45. (Withdrawn) The isolated nucleic acid molecule according to claim 44, wherein said mammalian guanylate kinase moiety is a murine guanylate kinase or a human guanylate kinase.

46. (Withdrawn) The fusion protein according to claim 42, wherein said mutant thymidine kinase is an enzyme comprising one or more mutations, at least one of said mutations encoding an amino acid substitution located toward the N-terminus from a DRH nucleoside binding site.



47. (Withdrawn) The fusion protein according to claim 42, wherein said mutant thymidine kinase is an enzyme comprising one or more mutations, at least one of said mutations being an amino acid substitution within a DRH nucleoside binding site.

48. (Withdrawn) The fusion protein according to claim 42, wherein said mutant thymidine kinase is an enzyme comprising at least three mutations, at least two of said mutations encoding amino acid substitutions that are located one, two or three amino acids toward the N-terminus from a DRH nucleoside binding site, and at least one of said mutations encoding an amino acid substitution that is located four or five amino acids toward the C-terminus from a DRH nucleoside binding site.

49. (Withdrawn) The fusion protein according to claim 46, wherein said mutant thymidine kinase is an enzyme comprising at least one mutation in the Q substrate binding domain.

50. (Withdrawn) The fusion protein according to claim 42, wherein said thymidine kinase is selected from the group consisting of Herpes Simplex Virus Type 1 thymidine kinase and Herpes Simplex Virus Type 2 thymidine kinase.

51. (Withdrawn) A method of inhibiting a pathogenic agent in a warm-blooded animal, comprising administering to a warm-blooded animal a vector according to claim 27, such that said pathogenic agent is inhibited.

52. (Withdrawn) The method according to claim 51 wherein said vector is administered *in vivo*.

53. (Withdrawn) The method according to claim 51 wherein said pathogenic agent is selected from the group consisting of viruses, bacteria and parasites.

54. (Withdrawn) The method according to claim 51 wherein said pathogenic agent is a tumor cell.

55. (Withdrawn) The method according to claim 51 wherein said pathogenic agent is an autoreactive immune cell.

56. (Withdrawn) The method according to any one of claims 51 to 55, further comprising the step of administering a nucleoside analogue.

57. (Withdrawn) The method according to claim 56 wherein said nucleoside analogue is selected from the group consisting of ganciclovir, acyclovir, trifluorothymidine, 1-[2-deoxy, 2-fluoro, beta-D-arabino furanosyl]-5-iodouracil, ara-A, araT 1-beta-D-arabinofuranoxyl thymine, 5-ethyl-2'-deoxyuridine, 5-iodo-5'-amino-2,5'-dideoxyuridine, idoxuridine, AZT, AIU, dideoxycytidine and AraC.

58. (Withdrawn) A pharmaceutical composition, comprising a vector according to claim 27, and a pharmaceutically acceptable carrier or diluent.

59. (Withdrawn) A pharmaceutical composition, comprising a host cell according to claim 29, along with a pharmaceutically acceptable carrier or diluent.

60. (Withdrawn) A method for monitoring the progress of gene therapy in a subject who has received a vector comprising a nucleic acid molecule of either claim 1 or claim 2 that encodes a *Herpesviridae* thymidine kinase enzyme and a radiolabeled anti-viral drug that is a substrate for said thymidine kinase, comprising the step of scanning said subject for the presence of said radiolabeled anti-viral drug.

61. (New) An isolated nucleic acid molecule encoding a *Herpesviridae* thymidine kinase enzyme of SEQ ID NO:1 comprising at least two mutations relative to SEQ ID NO:1, wherein said isolated nucleic acid molecule encodes a thymidine kinase enzyme comprising SEQ ID NO:107 or SEQ ID NO:108, wherein said SEQ ID NO:107

or SEQ ID NO:108 comprises amino acid substitutions for wild type amino acids at positions 159-161 and 168-169.